Cytochrome CYP450 reaction phenotyping

Background: Cytochrome P450 (CYP) enzymes represent a heme-containing protein superfamily metabolizing a broad variety of xenobiotics, including drugs and toxic chemicals. Eleven CYP families are expressed in human liver and gastrointestinal tract (CYP1A2, CYP2A6, CYP2B6, CYP2C8/9/18/19, CYP2D6, CYP2E1, and CYP3A4/5), and 5 of them (CYPs 1A2, 2C9, 2C19, 2D6 and 3A4) are involved in about 95% of the known drug metabolism. Cytochrome P450s are of critical importance due to the two of the most significant issues in clinical pharmacology: metabolism-mediated drug-drug interactions and individual variability in drug metabolism (CYP450 gene polymorphism). CYP450 reaction phenotyping involves the identification of enzymes responsible for metabolism of the test article, which is useful for prediction of drugdrug interactions (DDI) and is recommended by the FDA. In case when a new drug candidate is metabolized by more than one isozyme, blocking one metabolic pathway by co-administered inhibiting drug engender metabolic switching to an alternative uninhibited enzyme.

Service Details: In this assay two metabolically active test systems are used: individual human recombinant cytochromes (CYPs 1A2, 2C9, 2C19, 2D6 and 3A4) and human liver microsomes. The test compound is incubated with each CYP450 isoform and the metabolising ability of the enzyme is estimated by disappearance rate of parent drug using HPLC-MS/MS analysis. Reference cytochrome specific substrates are used as controls. In the microsomal assay the isoform-specific CYP450 inhibitors are used, and the increase of the half-life (t1/2) in the presence of the inhibitor indicates which enzyme is responsible for the metabolism of the compound.

CYP450	Substrate (reference)	Inhibitor	Inhibitor concentration, uM
1A2	Phenacetin	α-Napthoflavone	1
3A4	Testosterone	Ketoconazole	1 and 10
2C9	Diclofenac	Sulphaphenazole	10
2C19	Omeprazole	Ticlopidine	20
2D6	Dextromethorphan	Quinidine	1

Deliverable: Metabolic stability data of 1 test article and 5 reference compounds (Phenacetin, Testosterone, Diclofenac, Omeprazole, and Dextromethorphan) in the presence of individual human recombinant cytochromes at tree time points over 60 minutes. Metabolic stability data for 1 test article in human liver microsomes in the presence of specific cytochrome inhibitors responsible for its metabolism (identified in the study with recombinant enzymes) at five time points over 60 minutes obtained by HPLC-MS/MS analysis. Data include parent compound percent remaining, half-life and clearance values. Full study report is provided.

Sample Submission: A minimal accurately weighable quantity of dry compound (~1 mg or 2 µmol) or 50 µL of 20 mM stock DMSO solution is required for this assay. For multiple assays, lesser amount of compound per assay may be sufficient, depending on the particular project. We do not need to know structures of the molecules for ADME testing. However, brutto formulas have to be provided for all studies involving MS detection.